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Primary Thrombophilia in Mexico: A Prospective Study

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A group of 102 Mexican Mestizo patients with appropriate clinical features suggestive of primary thrombophilia was prospectively studied. Thirty-nine percent of them had activated protein C resistance, but only four patients displayed the factor V Leiden mutation. Five percent of the individuals were found to be protein C deficient, whereas 2% had protein S deficiency. No cases of abnormalities in antithrombin III, plasminogen, tissue-type plasminogen activator or plasminogen activator inhibitor were found. The low prevalence of the activated protein C resistance genotype, probably stemming from the genetic admixture of the Mexican Mestizo group is noteworthy. Am. J. Hematol. 60:1–5, 1999.

Key words: thrombophilia; thrombosis; protein C; protein S; protein C resistance; factor V Leiden; Mexico

INTRODUCTION

The past few years have witnessed salient advances in our knowledge of inherited thrombotic disorders [1]. Until recently, more than 70% of patients with appropriate clinical features for inherited thrombosis and no identifiable risk factors were termed ‘‘idiopathic.’’ The most significant advance in the understanding of the hereditary thrombophilia has been the identification of activated protein C resistance (APCr) as a common thrombotic disorder [1–3]. In Caucasian populations and in patients with clinical features for inherited thrombophilia, primary APCr is found in 20–60% of cases, protein C (PC) deficiency in 5–6%, protein S (PS) deficiency in 5–6%, antithrombin III (AT-III) deficiency in 1–2%, and other conditions (heparin cofactor II deficiency, dysfibrinogenemia, plasminogen deficiency, tissue-type plasminogen activator (TPA) deficiency, excess TPA inhibitor (PAI) activity, homocysteinemia, etc.) in less than 2% of cases [1]. Accordingly, molecular studies combined with laboratory techniques allow accurate classification of at least half of Caucasian patients with inherited thrombotic disorders. Since there are data suggesting variations in the world distribution of certain hereditary thrombophilic conditions [4], and considering that the genetic composition of the Mexican Mestizos has been shown to have 56% of Indian genes, 40% of Caucasian genes, and 4% of black genes [5], we decided to analyze prospectively Mexican Mestizo patients with clinical features for inherited thrombophilia, in order to assess the prevalence of the primary thrombophilic conditions.

MATERIAL AND METHODS

Patients

Over a 36-month period, all consecutive Mexican Mestizo patients referred to our clinic by physicians from different parts of the country were prospectively accrued in the study if they had one of the following clinical markers associated with a primary hypercoagulable state [6,7]: 1. Thrombosis at an age below 40 years; 2. Family history of thrombosis; 3. Recurrent thrombosis without apparent precipitating factors; 4. Thrombosis at unusual anatomic sites; and 5. Resistance to conventional antithrombotic therapy. Individuals with systemic lupus erythematosus, secondary or primary anti-phospholipid syndrome, overt malignancy, puerperium, those who were

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pregnant or taking oral contraceptives, or had other conditions associated with secondary thrombophilia were excluded from the study.

Analytical Methods

APCr phenotype. Functional resistance to activated PC in plasma was measured using the test kit Acticlot aPC (American Diagnostica, Greenwich, CT). aPC resistance was determined by measuring the prolongation of activated partial thromboplastin time (aPTT) in response to aPC. It was considered positive when the ratio of aPTT clotting time of the sample in the presence of APC/sample without APC was less than 2.1 [2,3,8]. The modification of the test according to the suggestion of Jorquera et al. [9], using factor V-deficient plasma was used in the last 16 cases of functional APC resistance.

APCr genotype (factor V Leiden mutation). In cases with the APCr phenotype, a polymerase chain reaction (PCR)-based analysis for the factor V R-506-Q gene mutation was performed according to Zöller and Dahlbäck [10]. Briefly, a region of the factor V gene comprising nucleotides 1690 to 1692 of the codon 506 was amplified by PCR and then subjected to digestions with restriction endonuclease Mnl. The restriction pattern was analyzed by electrophoresis on a 4.5% polyacrylamide gel. The oligonucleotides used are described in the article by Zöller and Dahlbäck [10].

Coagulation protein C. Functional anticoagulant PC levels were measured by the method of Francis and Seyfert [11], using the venom of the copperhead snake *Agkistrodon contortrix* to activate PC in the plasma sample and using an end-point chromogenic substrate (H-D-Lys (Cho)-Pro-Arg-pNA.2AcOH) system as indicator (Spectrozyme PCA, American Diagnostica). Deficiency was defined at levels below 60%, with normal range of 60–140%.

Coagulation protein S. Free protein S and C4bp-bound protein S were assessed according to Comp et al. [12] using Laurell's rocket electrophoresis of the plasma and of the supernatant after precipitation in polyethylene glycol. Deficiency was defined at levels below 60%.

AT-III. Laurell's rocket electrophoresis [13] was used to measure antigenic AT-III. Functional AT-III was measured according to Frantzen et al. [14], using a chromogenic substrate (Berichrom AT-III).

Plasminogen. It was assessed by a chromogenic substrate (H-D-N1e-HHT-Lys-pNA.2AcOH) assay, using streptokinase as activator, according to Soria et al. [15]; (Actichrome PLG, American Diagnostica). Normal ranges are 60–140%.

Tissue-type plasminogen activator activity. It was evaluated in unstimulated plasma, by the method of Chmielewska et al. [16], using a chromogenic substrate (CH3SO2-D-CHT-Gly-Arg-pNA.AcOH) and physiologic fibrin stimulation (Spectrolyse/Desafib, American Diagnostica). Normal ranges are 0.01–0.8 IU/ml.

Plasminogen activator inhibitor activity. Using the same chromogenic substrate for TPA as end point, the assay with physiologic fibrin stimulation was done simultaneously with the TPA assessment [16] (Spectrolyse/Desafib, American Diagnostica); normal ranges have been determined between 0–6 IU/ml.

Plasminogen activator inhibitor type 1. Its antigenic levels were assessed using a double antibody enzyme immunoassay (ELISA), using two different monoclonal antibodies, to detect both active (free) and inactive forms of PAI-1, according to Urden and Blömback [17] (Imubind-1 PAI-1, American Diagnostica). Normal values were 1.53–3.87 ng/ml.

Anti-phospholipid antibodies (APLA). Both IgG and IgM isotypes were determined in serum by a solid phase immunoenzymatic assay (ELISA) using cardiolipin as antigen as previously described [1,18]. Known positive and negative sera for standardization were kindly provided by Dr. D. Alarcón-Segovia, México City. Normal values were determined in our laboratory as follows: IgG below 1.9 and IgM below 2.4 standard deviations (SD) above the value observed in a pool of 10 normal plasmas [19,20].

Lupus anticoagulants (LA). These were assessed by a simplified single-vial dilute Russell's viper venom time test (DVVtest, American Diagnostica) [21]. A test result within mean value ± 2 SD of our laboratory-established normal reference range was considered as negative for LA. The ratio of clotting time of a 1:1 mixture of test and normal plasma divided by the clotting time of pooled normal plasma, greater than two SD was considered as positive for LA.

RESULTS

We started this prospective study when the tests to assess the APCr were available in our clinic and performed on a routine basis in thrombophilic patients; accordingly, 102 consecutive patients were accrued in a 36-month period. The median age of the patients was 41 years (range 11–83 years); there were only two patients aged less than 18 years. In 47 patients (46%) the results of all the tests were found within the normal range. The APCr phenotype was found in 40 patients, PC functional deficiency was found in five patients, PC antigenic deficiency was present in one of the five patients with functional PC deficiency, and in two patients abnormally low levels of PS were found. In one patient abnormally low levels of functional PC were found in conjunction with the APCr phenotype. No cases of abnormalities in AT-III, plasminogen, TPA, or PAI were found. Hyperhomocystinemia/homocystinuria was not looked for. Table I summarizes the findings identified in this cohort.

TABLE I. Thrombophilic Conditions Identified in the Cohort of Mexican Mestizo Individuals With a Clinical Marker Suggestive of an Inherited Hypercoagulable State

Activated protein C resistance	39.2%
Protein C deficiency	4.9%
Protein S deficiency	1.9%
Others	0%

DISCUSSION

It is noteworthy that the most frequent abnormality was the APCr phenotype (40 cases) which represents 39.2% of cases with a clinical marker of familial thrombophilia. Interestingly, the APCr phenotype associated with the factor V Leiden mutation was found only in 10% of the patients with the APCr phenotype which is 4% of the patients with thrombophilia (4 patients). This finding contrasts with data from Caucasian populations in which patients with clinical features for inherited thrombophilia are found to have the factor V Leiden mutation in 20–60% of the cases [1]. Preliminary data have shown that the prevalence of the factor V Leiden mutation is low in Mexican Mestizos [2,22–23] and absent in some Mexican Indian groups [24], confirming the observation that the mutation in the factor V gene (G→A, 1691) leading into the (R 506 Q) mutation that produces a mutated factor V has a geographic distribution and is genetically related to Nordic Caucasians [4,25]. Since the Caucasian component of the Mexican Mestizos is low [5,26], a low prevalence of the APCr genotype was expected. In one patient with severe thrombophilia, the APCr phenotype was associated with PC deficiency; the family study of this case has shown that the combined thrombophilic conditions are not associated with the factor V Leiden mutation [2] and studies are being conducted to define the inherited abnormality leading into the APCr phenotype observed in this family.

Most cases of the APCr phenotype found in this cohort were either acquired or not related to the R 506 Q factor V mutation. APLA and LA are relatively common causes of acquired forms of APCr [2,23,27–29]. In four of these cases APLA or LA was found. We have previously shown that autoimmune conditions associated with the presence of either APLA or LA are frequently associated with acquired forms of APCr [27]. In fact, up to 50% of patients with autoimmune disorders displaying APLA or LA may show the APCr phenotype [27,29]. Since the prevalence of autoimmune disorders is higher in Mexican Mestizos than in Caucasian populations, probably stemming from the genetic admixture between Amerindians and Caucasians [30], it is therefore possible that individuals with subclinical forms of autoimmune disorders could have been included in this cohort in which individuals with overt autoimmune disorders were excluded. In this subset of patients, the values of the aPTT were not prolonged.

To overcome the in vitro effect of APLA or LA in the assessment of the APCr phenotype, modifications to the original method have been suggested [9]. We assessed the APCr phenotype using this modification in the last 16 cases of the patients with APCr phenotype identified in this series and found an abnormal result in six of them (37%), a figure similar to that found in the rest of the group. In this subset of six patients, only one patient was identified with the APCr genotype. It is obviously possible that undetectable autoantibodies could be somehow related to the abnormal results found in some of the cases with the APCr phenotype lacking the APCr genotype. It may also be true that some of the patients could have ‘‘spurious’’ APCr phenotype [31] if the modified test using factor V-deficient plasma [9] would have been used. Since patients with either primary or secondary forms of the antiphospholipid syndrome may suffer several acquired thrombophilic abnormalities [19–20,23,27–29,32–36], they were specifically excluded from this cohort. Other conditions leading into acquired forms of APCr such as liver disease, pregnancy, or contraceptive use [2] were ruled out by the physicians referring the patients, but cannot be absolutely excluded. It may also be that other factor V gene abnormalities different from the R506Q and leading into the APCr phenotype could have been present in some of the cases [37]. Perhaps the low prevalence of the mutation in the factor V gene (G→A, 1691) leading into the (R 506 Q) mutation identified in this series of thrombophilic patients explains that no cases of cosegregation of factor V Leiden with AT-III, PC, or PS deficiencies were identified.

The first case of inherited PC deficiency in Mexico was identified in 1987 [38]; it was a case of solely functional type II deficiency [38]. Since most (4/5) cases of PC deficiency in this cohort were purely functional, it would seem that the assay to measure the function of the protein would be the initial step in the study of this condition, in Mexico. The family that displayed both type II functional PC deficiency and APCr without the factor V Leiden mutation is noteworthy: Only one male of four siblings with the combination of these two thrombophilic conditions has severe thrombophilia, whereas the remaining three are asymptomatic [2]. Both cases of PS deficiency belonged to the type I (total, free, and C4bp-bound PS) [39].

In summary, in a group of Mexican Mestizos displaying clinical markers of primary thrombophilia, we have found APCr as the most frequent condition; however, inherited forms of the disorder seem to be less frequent as compared with Caucasian populations [1,40]. Other thrombophilic conditions such as PC and PS deficiency were identified in proportions similar to those described in Caucasians [1]. The differences may be related to the ethnic composition of the Mexican Mestizo group or to

other nonidentified conditions probably related to the concept of geographic hematology [41].

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